

# Cardiohistological Changes in Rats From Single Episodes of Maintained Forced Exercise

S. T. RAPUNDALO,<sup>1</sup> M. A. PERSINGER AND M. A. ALIKHAN

Departments of Biology and Psychology, Laurentian University, Sudbury, Ontario, P3E 2C6, Canada

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RAPUNDALO, S. T., M. A. PERSINGER AND M. A. ALIKHAN. *Cardiohistological changes in rats from single episodes of maintained forced exercise*. *PHYSIOL. BEHAV.* 25(3) 433-438, 1980.—Groups of young and mature rats were exposed to a single episode of either forced running: 1, 6, 12 or 24 hr duration or to control (sedentary) conditions for similar durations. Both young and mature rats demonstrated highly significant increases in cardiac wet weights as a function of exercise duration. The (three) independent variables accounted for 80% of the variability in heart weights. Exercise-dependent and highly significant fuchsinophilic histological changes were noted in large sections of heart tissue proximal to the major blood vessels. Other sections of the heart did not demonstrate this effect significantly. Histological profiles indicate that acute maintained exercise of previously non-exercised rats can produce histological changes indicative of myocardial degeneration.

| Histology | Myocardium | Fuchsinophilia | Exercise | Rat |
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**PATHOLOGICAL** changes in heart tissue associated with forced exercise have been described by several authors [5, 6, 11, 24]. In rats, the most common consequence associated with forced exercise schedules has been cardiac hypertrophy as measured by the increased wet weights [2, 3, 8-10, 13, 15, 16, 18-20]. However all of these studies involved repeated exercise training or periodic exposures to brief exercise intervals. None of these studies investigated histological changes.

The possibility that singular (acute), maintained exposures to forced exercise regimes could produce discernable histological alterations in heart tissues has not been investigated thoroughly. This study was designed to answer two specific questions: (1) are there systematic increases in cardiac hypertrophy following single, maintained exercise intervals, and (2) are discernable, histological changes evident that could suggest tissue damage.

## EXPERIMENT 1

### METHOD

#### Animals

Twenty-four 65 day-old and 24 one-year-old (retired breeders) female albino Wistar rats obtained from Biobreeding Laboratories, Ottawa, Ontario, Canada, were selected as subjects.

#### Procedure

Following arrival from the breeding laboratory, pairs of

rats were housed in standard wire (41×24×18 cm) cages and maintained at least 14 days within normal colony conditions: temperature  $24 \pm 1^\circ\text{C}$ , relative humidity  $45 \pm 5\%$  and 12:12 LD (light dark) regiment. Food and water were available ad lib.

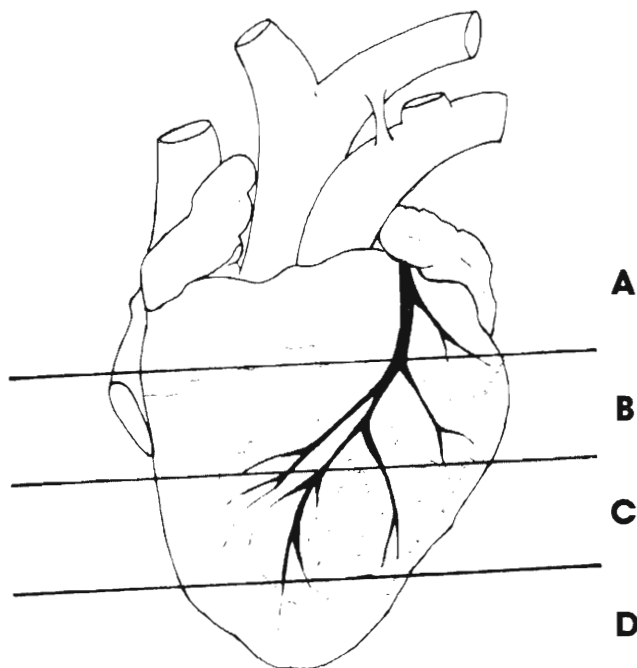
Over a 20 day period, young and mature rats were weighed and then were allocated to one of four running time conditions or to one of four control conditions. The four running time conditions were: 1 hr, 6 hr, 12 hr and 24 hr. Rats in these conditions were placed in a standard Gebrands running wheel ( $r=17$  cm) driven continuously at 6 rpms by a Graham variable speed transmission motor and were maintained there for the duration of the test periods. Periodic checks were made to insure the animals maintained ambulatory activity (and had not been fatigued to the point of rolling inside the wheel).

Since the wheel was turning continuously, no food or water were available to the experimental subjects during the test periods. Control subjects were maintained in 38×24×18 cm plastic cages (food and water ad lib) for the appropriate control period. The control cage was placed within 0.5 m of the test apparatus in order to control for noise and possible situation effects.

A total of 8 subjects (4 mature and 4 young) were tested in each of the 4 running times and a total of 4 subjects (2 young and 2 mature) were maintained in each of the four control conditions. The serial order of the experimental and control condition was randomized over the 20 day period. Termination of these conditions ranged between 0800 and 1800 hr.

After the appropriate period of time, experimental sub-

<sup>1</sup>S. T. Rapundalo is now at: Department of Physiology, Box 551 MCV Station, Medical College of Virginia, Richmond, Virginia 23298, U.S.A.



## RAT HEART: Primary Sections

FIG. 1. Section designation for histological analyses.

jects were weighed and killed by decapitation. After allowing trunk (body) drainage, the hearts were removed by cutting the greater vessels approximately 0.25 cm from the tissue base and weighed quickly. The heart tissues were then perfused with physiological saline and fixed in ethanol (90 pts 80% ethanol), Formalin (5 pts 30% formaldehyde), acetic acid (5 pts glacial) for 48 hr.

Each heart was sectioned into four parts (blocks) from apex to base in a plane parallel to the atrial ventricular groove (see Fig. 1). Following dehydration and clearing tissue blocks were infiltrated and embedded in paraffin. Each tissue block was microtomed completely into 6  $\mu$ m sections. Two non-adjacent 6  $\mu$ m sections from throughout each of the four blocks for each heart was stained with acid-fuchsin technique according to Bajusz [1] for fuchsinophilia.

The extent of myocardial degeneration, as indicated by fuchsinophilia (FP) in each section was evaluated using the following rating scale: 0 (no FP), 1 (few small focal areas of FP), 2 (FP of 25% of the myocardium), 3 (FP over 50% of the myocardium) and 4 (FP of 75% of the myocardium). All slides were evaluated on three separate two hour occasions during which time all 384 slides were rated. The average of the three ratings was calculated as each section's score.

Three-way analyses of variance according to age (young vs mature), experimental treatment (forced run vs controls) and time of exposure (1 hr, 6 hr, 12 hr, and 24 hr) for body and tissue measures and for histological ratings were completed by computer and checked manually. The three-way interactions were not calculated because the number of subjects were considered too small. Non-parametric analysis ( $\chi^2$ ) were run on histological rating data.

### RESULTS

Means and standard errors for absolute cardiac weights

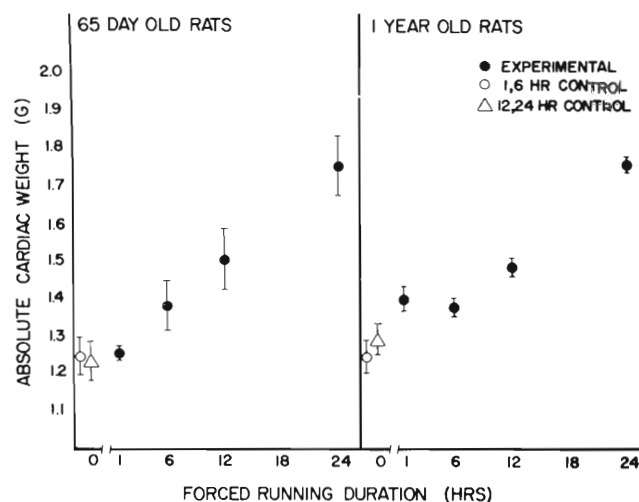


FIG. 2. Absolute wet heart weights for experimental rats following various durations of forced exercise in a running wheel and for control rats exposed to sedentary conditions. Vertical bars indicate standard error of the mean ( $n=4$ /group).

according to age, experimental condition and exposure time are presented in Fig. 2. Three-way ANOVA demonstrated significant differences between the forced-exercise and sedentary controls  $F(1,35)=43.65$ ,  $p<0.001$ , and between the four exposure times  $F(3,35)=16.01$ ,  $p<0.001$ . A significant  $F(3,35)$   $p<0.001$  experimental condition by the run time interaction was evident; all other differences were not significant.

Ad hoc Scheffes test set at  $p<0.05$  indicated that the 12 and 24 hr young exercised groups had significantly heavier heart weights than either the controls, 1 hr or 6 hr exercised groups. Similarly, for the older rats, the 12 hr and 24 hr exercised groups displayed significantly heavier heart weights than the other groups. Omega-square estimates showed that run time accounted for approximately 35% of the heart weight variability. The total explained variance was calculated to be 80%.

Figure 3 demonstrates the percent body weight loss during the exposure periods according to age and condition. Three-way ANOVA indicated significant run time  $F(3,35)=9.83$ ,  $p<0.001$ , age  $F(1,35)=10.06$ ,  $p=0.003$  and experimental condition  $F(1,35)=56.94$ ,  $p<0.001$  differences. Both run time by condition  $F(3,35)=5.95$ ,  $p<0.001$  and age by condition  $F(1,35)=14.31$ ,  $p<0.001$  interactions were significant. All other differences were not significant.

Ad hoc Scheffe's (set at  $p<0.05$ ) for the young rats indicated that each of the three groups with run times greater than 1 hr were significantly different from each other and from the 1 hr and control groups. For the mature rats, the 12 and 24 hr groups lost more weight than the 6 hr group which in turn lost more weight than the 1 hr or control groups. Run time accounted for 20% of the variance while the total explained variance approached 80%.

Chi-square analysis as a function of the 4 run times (two conditions) on the 4 histological ratings indicated a significant effect  $\chi^2(7)=35.74$ ,  $p<0.001$  for the A-level (base of the ventricle) only. Further  $\chi^2$  analyses indicated no significant differences for the other three sections or between ages. Figure 4 shows the means and standard errors for fuchsinophilic ratings for each of the four heart sections as a function of run time for the exercised groups and sedentary controls (1+6 hr and 12+24 hr).

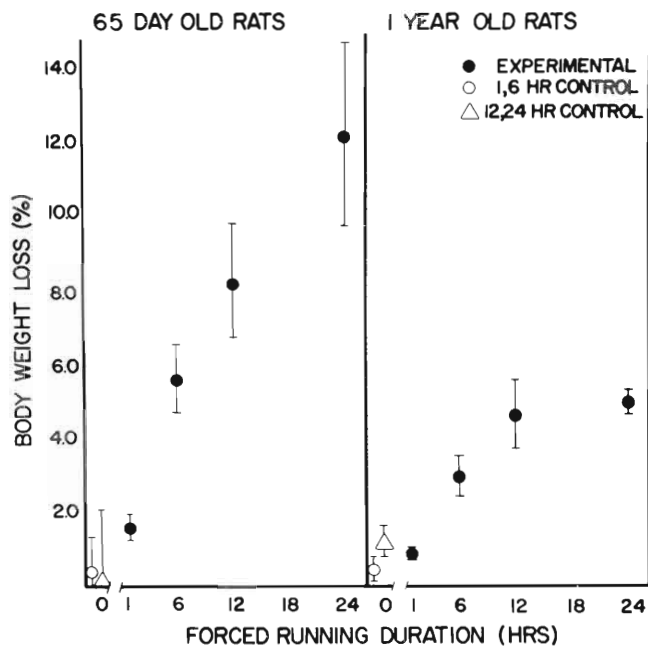


FIG. 3. Percent body weight loss following various durations of forced exercise in a running wheel (experimental rats) or no forced exercise (controls). Vertical bars indicate standard error of the mean ( $n=4/\text{group}$ ).

Since the ordinal scores for the A-level approached normality, a three-way ANOVA was conducted. Again the hearts of animals that were exposed to the protracted running showed significantly more fuchsinophilia  $F(1,35)=25.57, p<0.001$ . The increase with running time was marginally significant  $F(3,35)=3.00, p<0.05$ . The main effects accounted for 41% of the variability; total explained variability was 60%. Sample photographs of the fuchsinophilic response in heart tissue from rats exposed to different experimental conditions are shown in Fig. 5.

## EXPERIMENT 2

Although the reliability of the increased heart weights was indicated by both the replication across groups (ages) and the duration-dependent nature of the weight changes, the possible confounding consequences of the mandatory food and water deprivation during the forced running were still present. Previous studies by Highman and Atland [7] have clearly demonstrated that several blood and cardiohistological measures did not differ significantly between rats that were housed for 6 hr with or without food and water (although forced exercise did produce discernable changes). In the present study, three conditions: (1) the cage controls (food and water ad lib), (2) 24 hr forced-exercise, and (3) running wheel control (no food and water and maintained within a non-operating running wheel for 24 hr) were compared.

## METHOD

### Animals

Eighteen 6 month old (retired breeder) female albino Wistar rats were used as subjects.

### Procedure

Over a week period, rats were allocated to one of three

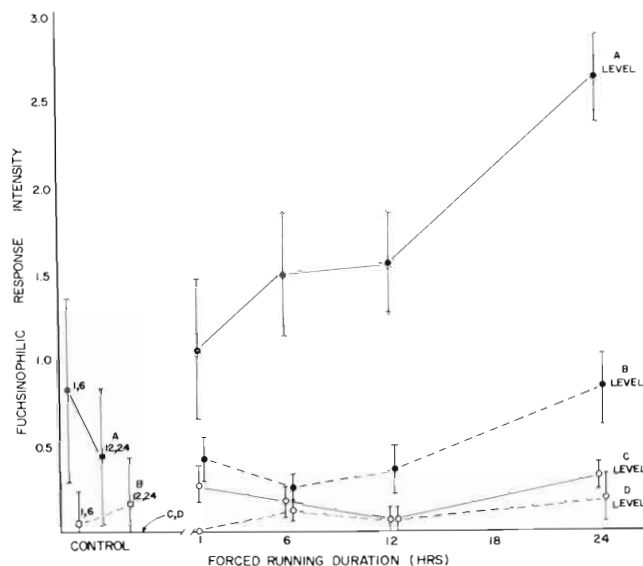


FIG. 4. Fuchsinophilic intensity for different sections (A, B, C, and D levels) of rat hearts (from Fig. 1) as a function of forced exercise (circles) or control conditions (squares). Note: No discernable fuchsinophilia was noted in C and D sections from the control rat hearts.

conditions: (1) cage control (as in Experiment 1, food and water ad lib), (2) 24 hr forced-running schedule (as in Experiment 1) or (3) running wheel control. The rats within the running wheel control condition were maintained without food or water within the wheel portion of the running wheel (not connected to any motor).

After 24 hr of exposure to the conditions, the forced exercise and two control groups were killed by decapitation. Hearts were weighed and fixed following previous procedures. Two sections from each of the four major heart sections were stained for fuchsinophilia; the average rating for the two slides was used as the animals' score for that section. All analyses were completed by computer.

## RESULTS

The results were comparable in both absolute values and magnitude of differences with those of Experiment 1. The means and standard errors of the mean (SEM) for absolute wet heart weights for the cage control (food and water ad lib), running wheel control (no food and water for 24 hr) and 24 hr forced-running groups were  $1.34 \pm 0.10$ ,  $1.32 \pm 0.12$ , and  $1.74 \pm 0.07$ , respectively,  $F(2,15)=581, p<0.001$ . Ad hoc tests indicated no significant differences between the two control groups.

The mean ( $\pm$ SEM) percent weight losses during the 24 hr exposure to the experimental conditions for the cage control, running wheel control and forced exercise groups were  $0.04\% \pm 0.27$ ,  $2.26 \pm 0.43\%$  and  $11.05 \pm 1.0\%$ , respectively,  $F(2,15)=87.21, p<0.001$ . Again, only the forced exercise group was the source of statistical significance. Histological results demonstrated fuchsinophilic patterns that were almost identical to Experiment 1. The most significant source of differences between the three groups occurred in section A. Mean ( $\pm$ SEM) fuchsinophilic scores for the cage control, wheel control and forced-exercise groups were:  $1.00 \pm 0.00$ ,  $1.08 \pm 0.20$ , and  $2.67 \pm 0.21$ , respectively,

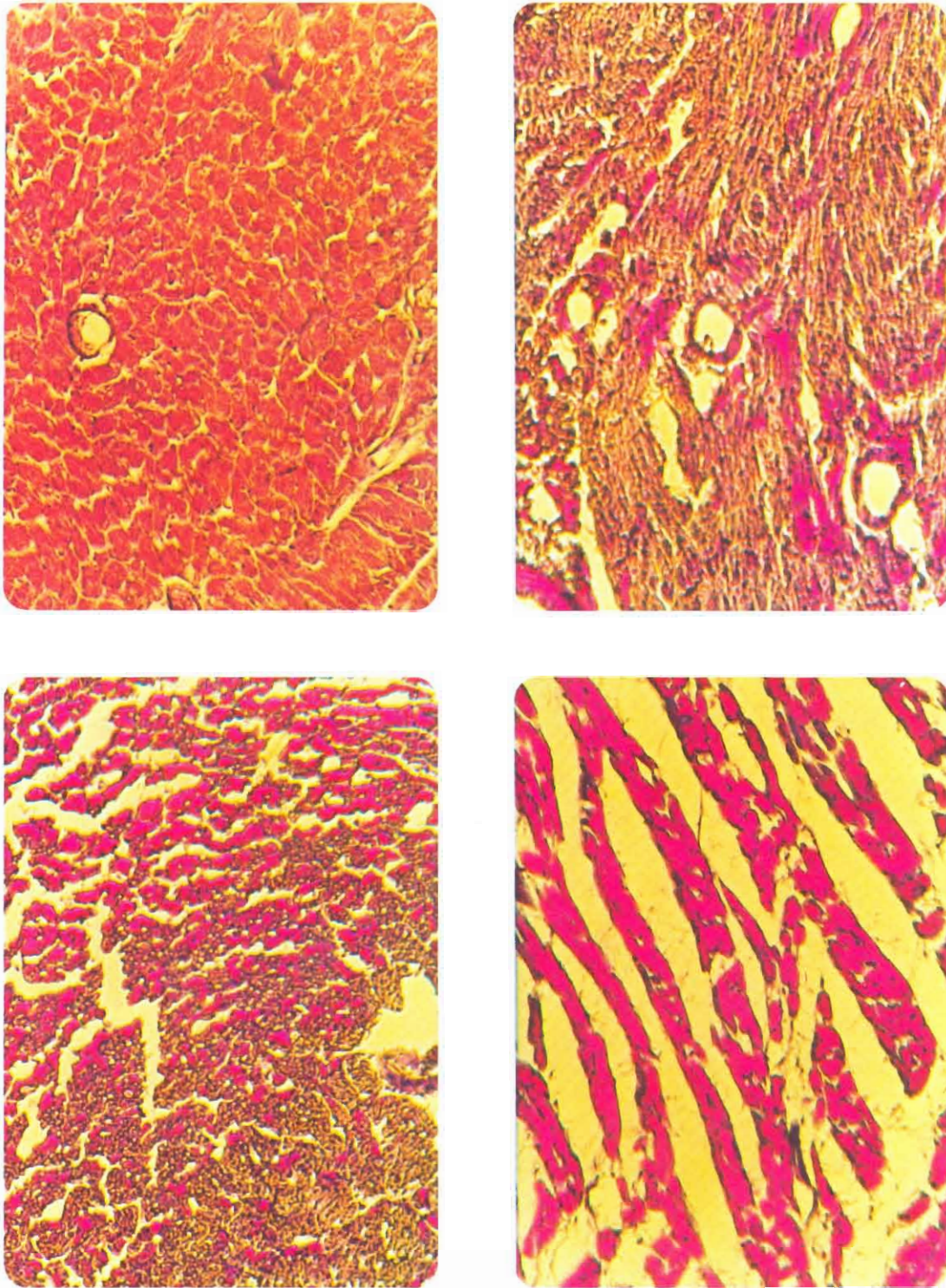


FIG. 5. Sample photographs of microscopic sections ( $75\times$ ) from Level A of heart tissues stained with acid fuchsin. Durations of forced exercise are specified. The intense purplish-red coloration is considered a fuchsinophilic response over the yellowish background. A: sedentary (6 hr) control, B: 1 hr exercise, C: 6 hr exercise, D: 24 hr exercise.

$F(2,15)=31.23$ ,  $p<0.001$ . Mean fuchsinophilic scores on all other sections were less than 1.5 (for the forced-run group) and less than 0.5 for the two control groups.

## DISCUSSION

The experiments clearly demonstrated that single exposures to various periods of forced, maintained running wheel activity are associated with significant increases in both cardiac wet weight and fuchsinophilia. Both effects increased with the duration of running time that ranged from 1 hr to 24 hr. Sedentary controls that were maintained within ordinary cages but adjacent to the running apparatus did not display these effects. Even 24 hr of food/water deprivation and sedentary maintenance within the wheel did not significantly alter the cardiac measures from other control values.

Since the maximum duration of forced exercise time was 24 hr, the increased heart weight is most likely due to the accumulation of fluids within heart muscle and not to the growth of muscle fibers. The 40% increased heart weights for the 24 hr animals relative to the controls for both mature and young groups can be considered a traumatic cardiac condition. This statement is supported not only by the fuchsinophilic responses but also by the enhanced waviness and intermuscle space noted over large areas of the tissue. Similar changes have been associated with excessive stretching of non-contractile fibers during the bulging of ischemic tissues [17].

Fuchsinophilia has been considered a histological measure of early stages in myocardial degeneration. According to Poley, Fobes and Hall [12] and to Selye [14], slight fuchsinophilia may indicate reversible ischemic changes in myocardium while moderate to marked fuchsinophilia represents irreversible ischemic necrosis consistent with myocardial infarction. If these statements were generalized to the present data, then single exposure to forced exercise between 12 and 24 hr in duration procedures irreversible necrosis in about 50% of the dorsal one-fourth of the myocardium in rats.

A less radical conclusion would indict the validity of

fuchsinophilia as a measure of cardiac damage. The actual correlation between cardiac hypertrophy (wet weights) and fuchsinophilic scores for the ventricular section of the heart were between +0.40 and +0.30 in the 3 age groups of rats. Although these values may suggest that the degree of cardiac hypertrophy is not related strongly to fuchsinophilia one could argue as well that the limit lay in the ordinal scaling for the histological measure rather than in the technique. Controversy in the literature does persist. Whereas Conner [4] has argued in favor of the fuchsinophilic measure for heart damage, other experimenters have reported opposite opinions [21-23].

The propensity for the heart tissue most proximal to the major blood vessels (the A-level in this study) to display fuchsinophilic responses is demonstrated by the control groups. All four control groups (the 3 food/water ad lib, as well as the water/food-deprived, running wheel controls) demonstrated higher fuchsinophilic scores in this region than in the other three heart sections. With such proclivity to stain, one is not quite sure whether the high fuchsinophilia scores represent a severe alteration in tissue function.

The predominance of fuchsinophilia in a heart tissue adjacent to major vasculature and not around other heart sections, has both practical and theoretical implications. Procedures involving random or nonchalant selections of heart fragments following experimental exercise regimes might exclude the maximum areas of effect. Histological analysis of ventral regions of the heart in this study demonstrated no significant fuchsinophilic association to running time.

Regardless of the technical questions, the present study clearly demonstrates both wet weight and histological changes in rat hearts following acute forced exercise. Both the duration-dependent changes in the heart tissue weight and histology as well as the similarity between different age groups indicate the robust nature of the effects. Even if the fuchsinophilia is found to be tightly coupled to cardiac tissue edema and reversible fluid accumulations, the reliability of this simple and clear histological measure suggests its usefulness in behavioral-cardiovascular studies.

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